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Some features of qollen-formation in the Cucurbitaceae *

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(WITH PLATES 17–21)

Studies in the development and differentiation of the anther have been frequent in recent years, and in this line we may cite the researches of Gager,[9] Rosenberg,[22] Merrell,[15] and others. In all cases the primordium of the stamen appears as a slight elevation of the tissue which later becomes outwardly differentiated into its characteristic form and inwardly into spores, tapetum, endothecium, etc. Galinski [10] found that in certain of the grasses (*Secale*, *Triticum*) the anther-wall became differentiated into four layers, epidermal, fibrous, degenerating, and tapetal, and that the original pollen-mother-cells may divide several times. In *Zostera* as described by Rosenberg,[22] the elongated archesporial cells cut off from their ends the cells which form the tapetum. But in *Asclepias*,[9] *Silphium*,[15] and a majority of seed-plants, the immediate hypodermal layer has been shown to divide by periclinal walls to form the primary tapetal and primary sporogenous cells, the latter in some cases undergoing repeated divisions. But in the development of pollen, interest has centered chiefly in the mitotic features exhibited during the division of the pollen-mother-cells. Among these the origin of the spindle and the reduction and behavior of the chromosomes have received most attention. The results of these numerous studies have been well summed up by Davis,[7] and Coulter and Chamberlain,[6] and space will not be taken here for a similar task.

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* Contributions from the Botanical Laboratory of Syracuse University. I.

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But few plants of the *Cucurbitaceae* have received attention from this point of view. Mirbel[16] studied the development of the anther of *Cucurbita Pepo* and demonstrated the principal facts of the process. In small buds 2 mm. long he found no trace of the locules, but in a slightly later stage he was able to discern the spore-mother-cells and the tapetum. His figures of this condition represent the anther-wall, composed of four layers of cells under the epidermis including the tapetum. In buds 3 or 4 mm. long an additional layer of cells was detected in the anther wall. The development of the pollen-mother-cells, the formation of the tetrads, and the differentiation of the pollen-grain are well described and figured.

Naegeli[19] described certain features in the formation of pollen in *Cucurbita* and in *Bryonia dioica.* He seems to have observed the first division of the microspore-nucleus in *Cucurbita* but not to have interpreted correctly what he saw. He discusses more at length the differentiation of the exine and the behavior of the intine upon the germination of the grain.

To Warming,[25] however, we are indebted for an accurate study of the development of the anthers of *Bryonia alba* and *Cyclanthera pedata.* In both these cases the first periblem layer of the anther divides by periclinal walls. From the outer cells thus formed is developed the anther-wall by succeeding periclinal divisions, and the inner cells become the archesporium. In *Bryonia* the archesporium is a single layer of cells which later forms a mass of spore-mother-cells. Warming says that, as seen in transverse section, the pollen-mother-cells form one to several rows in each angle of the anther. Not all the cells cut off toward the inner side in the division of the hypodermal cell become mother-cells, and sometimes those that do become mother-cells do not divide again until the formation of the tetrads. In *Cyclanthera* the inner cells resulting from the division of the first periblem layer as a rule do not divide again but form mother-cells by growth.

Thus the evidence indicates that in these plants the usual order of development of sporogenous tissue in seed plants is adhered to, but the subsequent history of the archesporium may vary, as it appears at present, in accordance with the form and structure of the anther, in some cases the original archesporial cells remaining undivided, in others giving rise to a considerable mass of pollen-mother-cells.

In this study attention has been given to the development of the pollen in three species of the *Cucurbitaceae* representing as many genera. The forms studied are *Fevillea cordifolia* L., *Micrampelis lobata* (Michx.) Greene, and *Cyclanthera explodens* Naud.

In *Fevillea* at the time when the anther begins to form the archesporium appears. At this time the anthers are rounded bodies, more or less flattened laterally by mutual contact. Growth takes place more rapidly in a radial direction, and a little later by reason of lateral pressure they become almost triangular in cross-section. At the two angles of each anther on the periphery a group of cells retain their meristematic character while the rest become more vacuolated and react less strongly to stains.

In the two outer angles of each anther a layer of cells contiguous to the epidermis divides by periclinal walls, thus giving rise to the primary sporogenous and primary parietal cells. Both of these again undergo divisions. The parietal series gives rise to four layers of cells, so that the external wall of the anther early comes to consist of five layers of cells including the epidermis. In this region of the anther the greater number of sporogenous cells divide repeatedly so that an almost cylindrical mass of spore-mother-cells is developed in each angle of the anther.

As usual, the layer of sterile cells adjoining the sporogenous tissue is developed as a tapetum and presents the usual appearance of such a tissue. The cells of this layer have two or more nuclei each, and the size and chromatic content of these nuclei, as well as their deeply staining cytoplasm, mark them off in sharp contrast from the adjoining sterile tissue. The tapetal cells retain their appearance of activity almost to the time of maturity of the pollen.

The usual process takes place in the formation of microspores. These are enclosed for some time in a somewhat gelatinous envelope before they round off and develop the thick exine. The division of the nucleus of the spore takes place quite late if at all. Although almost mature anthers were sectioned, no case was found in which a microspore contained more than one nucleus.

In *Micrampelis* several cells immediately below the epidermis divide by periclinal walls. These cells are distributed along the ridges which later plainly mark off the positions of the locules. The series thus formed gives rise to three layers of cells, one of which forms the tapetum. Outside of the tapetum only three layers of cells including the epidermis are to be found in the mature anther.

The primary sporogenous cells enter directly upon a period of growth, becoming spore-mother-cells without divisions. The mother-cells form a linear series, sometimes broadening into a narrow plate but never dividing to form a cylindrical mass as in the case of *Fevillea*. These cells become large and prominent and each gives rise to four microspores. Here as in the preceding case the nucleus of the microspore does not divide to form vegetative and generative nucleus until quite late.

In *Cyclanthera* when the rudiment of the androecium has begun to assume the form of a disk the hypodermal cells prepare for periclinal divisions in two zones, one above the other. The cells in the two narrow bands thus situated divide as in the previous types to form the primary parietal and primary sporogenous cells (FIGURE 11). The former divide again to three layers, which with the epidermis constitute the outer wall of the locule (FIGURE 12). The primary sporogenous cells do not divide to increase the number of spore-mother-cells, but merely enter upon a period of growth until the time arrives for the formation of the macrospores. Four microspores are formed from each spore-mother-cell in the usual order. They remain for some time in a pyramidal arrangement surrounded by a transparent, seemingly gelatinous envelope, and during this time the exine begins to thicken and the nucleus assumes a less chromatic aspect. As they separate the microspores become rounded off and begin to develop their characteristic markings. Almost mature pollen-grains were observed in the sections, but each contained only one nucleus. The tapetum retains its active appearance until the spores are almost mature.

But the chief interest in pollen-formation in these forms is not in the facts just cited, but in the details of the process as they appear in the division of the mother-cells. In the few members of the *Cucurbitaceae* here under consideration, the course of development leading up to the formation of the pollen differs in no important particular. Some apparently minor differences have been noted which will be mentioned. The history of the microspores has been most carefully followed in *Micrampelis*, which is a favorable object for such a study. The principal stages in the formation of the microspore have also been noted in *Fevillea*, *Melothria*, *Cucurbita moschata*, *C. Pepo*, and *Cyclanthera*.

So far as the writer is aware no examination has been made of the cytology of the pollen-mother-cells of any of the *Cucurbitaceae*, though certain features of the vegetative cells have been studied by Zacharias.[27]

Though less favorable in some respects than some other subjects for such studies, certain features have been observed which seem worthy of attention.

The history of the microspore from this point of view begins with the formation of the primary sporogenous cell. At the conclusion of the mitosis which forms it, the sporogenous cell is not distinctly different in appearance from any of its hypodermal neighbors. A considerable number of those in its immediate vicinity have a decidedly meristematic character. At this time the cytoplasm of these cells is relatively thin as compared with the later states. The mitotic figure which is concerned in the first division of the hypodermal cell is a multipolar one, at least in the metaphase, and its chromatin is massed together in a compact zone at the equator.

The primary sporogenous cells become directly the pollen-mother-cells. They enter upon the growth period immediately after their formation, and soon become very conspicuous by reason of their size and structure. The expansion of their nuclei is the first distinctive feature shown in their growth, and this without any apparent increase in chromatic substance makes the nuclei appear quite clear, except for the single large nucleolus and their chromatin network. In appearance the cytoplasm consists of a very fine network of granular threads. The granules stain darkly and the cytoplasmic body has a grayish cast with the haematoxylon stain. At this time there is no perceptible arrangement of the cytoplasmic substance and it is equally dense in all parts. With the growth of the cell the granules increase in size slightly up to the time of the division. At about the time of synapsis the cytoplasm undergoes an interesting change. At this time the position of the nucleus in the cell is more or less eccentric and the nucleolus and the chromatin mass are usually on the side of the nucleus nearest the cell-wall. The cytoplasm, which up to this time has shown no special differentiation, now displays a number of fine fibers running tangent to the nucleus along the arc lying opposite the chromatin mass. These fibers may be traced distinctly to the periphery of the cytoplasm, where they appear to connect with the plasmatic membrane. The cell at this time presents an appearance much as if the nucleus by rapid expansion had placed under tension some of the fibers of the cytoplasmic reticulum (FIGURE 24). Toward the periphery of the cell an apparent branching of the fibers was often noticed and frequently thicker portions which stained darkly were plainly seen. If there is any significance in these fibers it is not apparent. As the nucleus resumes its position at the center of the cell at the close of the synapsis period they are no longer visible but the meshes of the cytoplasmic reticulum appear drawn out in a radial direction from the nucleus, which is a characteristic condition just preceding the prophase. There is, however, no indication of fibers such as appear in *Cobaea*,[13] *Larix*,[1] or *Equisetum*.[20] As these changes take place there is a drawing away of the ectoplasm or "*Hautschicht*" from the cell-wall, so that the mother-cells, instead of remaining angular, become practically spherical. Cannon[3] noted the same phenomenon in the spore-mother-cells of cotton and regarded it as the normal and regular process in those cells. The same conclusion seems to be justified here by the evidence at hand and by the subsequent history of the cells arising from the ensuing divisions. The spherical form is probably an advantage in the exact division of the cells into tetrads after the manner in which they usually occur. The nucleus, which in the earlier stages shows a perfectly even curvature of its membrane, now begins to contract and its outlines are less regular. This irregularity becomes more and more pronounced until the membrane is finally dissolved in the prophase of the first division.

In FIGURE 23 are represented certain cytoplasmic bodies whose history we have made an effort to follow. At a certain stage in the development of the mother-cells they are quite conspicuous even under a relatively low magnification (75 diameters). They are a series of short, crooked, darkly-staining fibers, which lie approximately parallel to one another on one side of the nucleus and about half way from the nucleus to the wall. They make their first appearance when the mother-cells are about half or two thirds grown and persist until the cytoplasm assumes the radial structure. As this change takes place they gradually disappear. About the time that the dark bodies disappear certain minute darkly-staining granules may be seen scattered promiscuously through the cytoplasm in its radial stage and during mitosis.

The rod-like bodies are remarkably constant features in *Micrampelis*, and appear when different staining reagents are used. It was first thought that they were portions of the nuclear chromatin which had been struck out into the cytoplasm in the process of cutting, but when it is observed that in the same section where many pollen-mother-cells are visible these bodies lie on all sides of the nuclei, such a conclusion as to their origin must be abandoned. Moreover in the same section some cells show them disposed horizontally, others show them in transverse section as a group of small black dots. It has been said that the fibers lie about parallel but occasionally they may assume a more or less radial arrangement around a certain point in the cytoplasm. They remain quite distinct up to the time when the cytoplasm begins to draw away from the cell-wall. The ends of these rods seem to weave in with the cytoplasmic meshes, especially toward the periphery of the cell where the reticulum is coarser. During the progress of the tetrad divisions the spherical, darkly-staining masses may be seen scattered through the cytoplasm, but near the close of the division they become clustered about the nuclei, and thus are divided among the microspores. They increase in size with the development of the spores and become very conspicuous until the pollen-grains near maturity when they gradually disappear. Bodies of an apparently similar nature have been found by Strasburger[24] in the pollen-mother-cells of *Larix*, and Allen[1], working upon the same subject, refers them to the class of extranuclear nucleoli and believes them to be proteid matters precipitated by fixing agents. But he finds them also in liquid surrounding the pollen-mother-cells, where they seem never to occur in *Micrampelis*.

The history of these bodies in *Micrampelis* is against such an interpretation, inasmuch as it would be difficult to account for their collection in the form described in a circumscribed portion of the cytoplasm. Moreover the change of form which they undergo seems to indicate that they are something more than mere passive by-products. Strasburger maintains that the cytoplasmic nucleoli bear an intimate relation to the kinoplasm, and supports his contention by citing the behavior of such bodies in *Larix* and other plants, in which they appear in connection with the spindle and other parts of the spindle-fibers and disappear at the conclusion of the division. He believes, however, that they are derived from the nucleolus, inasmuch as they appear as the nucleolus disappears and vanish again with its reappearance in the daughter-nucleus.

In this view Mottier[18] concurs, and chiefly on the basis of their staining reaction states that "there is no doubt that these bodies represent nucleolar substance." He suggests furthermore that the presence or absence of extranuclear nucleoli may depend upon the activity or condition of the cell, in view of the fact that they may be present in or absent from cells of the same tissue in the same stage of development. That the bodies here under consideration in *Micrampelis* are of the same nature as those described by Mottier in *Lilium* is difficult to say, though it seems a fair assumption that they are.

In *Micrampelis* no relation between these bodies and the nucleolus could be established. They appear in the cytoplasm long before the disappearance of the nucleolus and the nuclear membrane (FIGURE 24). That nucleolar material in solution might diffuse out through the nuclear membrane and be precipitated again in the cytoplasm is possible, but it seems highly improbable, and if so it might reasonably be expected to diffuse equally in all directions. In *Micrampelis*, however, the appearance of the darkly-staining granules is at first in a particular part of the cytoplasm and that the region occupied by the fibers above referred to. These cytoplasmic fibers seem similar to those observed by Duggar[8] in *Symplocarpus* and by Lloyd[14] in *Crucianella*. In neither of these works, however, does it appear that they bear any relation to the extranuclear nucleoli. Duggar refers to the almost simultaneous appearance of the fibers and darkly-staining granules, and regards both as due to the nuclear changes in synapsis. Lloyd regards the appearance of such cytoplasmic fibers as possibly due to the streaming of hyaloplasm or kinoplasm, from the fact of their similarity in appearance to features described by Wilson[26] and others working upon other material. That such a kinoplasmic streaming toward the nucleus occurs in *Micrampelis* is strongly suggested by the marked radial elongation of the cytoplasmic meshes during the prophase of the first division (FIGURE 25), but this feature is uniform throughout the cell.

A careful study of the material at hand has convinced the writer that in this case the extranuclear nucleoli arise from the conspicuous cytoplasmic fibers which make their appearance during the growth of the pollen-mother-cells. These fibers, at first thin and delicate, become thicker and stain more darkly, sometimes appearing as a series of nodules strung together, and again as thick crooked masses. As their development proceeds the fibers disappear and the round cytoplasmic nucleoli multiply and for a time appear in the area occupied by the fibers (FIGURES 23–68). It is recognized, however, that the phenomena observed may be open to more than one interpretation. The fibers observed lie in the center of the largest amount of cytoplasm in the cell and they must be taken as evidence of a cytoplasmic activity of some sort. It is possible that, these being the centers of special activity, they are the points at which the disposition of certain cytoplasmic products first takes place.

If we are to regard the spherical bodies in the cytoplasm of the spore-mother-cells of *Micrampelis* as extranuclear nucleoli, according to Strasburger's view, we must account for their persistence in the cytoplasm through all stages from one generation of cells to another. It does, nevertheless, seem that they are associated with the kinoplasm, inasmuch as they appear in the rod-like form to be a part of the cytoplasmic reticulum. That they are actually connected with the spindle at any stage of its development could not be determined accurately, though their position would often favor such a view. During the metaphase certain fibers may be seen straying out into the cytoplasm from the poles of the spindle, and seem to be in contact with the dark bodies scattered through it, and when at the conclusion of the division the contracting mantle-fibers have drawn the chromosomes to the poles and the daughter-nuclei have organized, the wandering fibers have also disappeared and the dark bodies have assembled about the nuclei. Hither, however, they may have been carried passively, as there is a congestion of the granular cytoplasm about the daughter-nuclei, leaving a comparatively clear broad zone across the equator of the spindle through which the division of the cytoplasm ultimately takes place.

With the organization of the tetrads the cytoplasm undergoes a change and instead of appearing fibrillar it begins to assume an alveolar aspect. It is during this stage that the activity of the cell is directed mainly toward the differentiation of the spore and the storage of a food reserve, which would, of course, account for the predominance of trophoplasm and the relatively slight quantity of kinoplasm present. As the spore enlarges the cytoplasm is distributed about its wall and only as it nears maturity does the central vacuole entirely disappear. The spore then becomes filled with granular proteinaceous matter. The differentiation of the exine begins while the tetrads are still clinging together, and is first evident by a thickening at the points where the germinal pores are afterwards to appear. In the mature spores, as usual, these are the thinnest places in the exine. The dark cytoplasmic bodies usually become segregated into some part of the spore as it approaches maturity and the cytoplasm in their vicinity usually stains more darkly than elsewhere.

Great interest centers in the behavior of the kinoplasm during the process of cell-division, and Strasburger[24] has given careful consideration to the various expressions of its activity, showing that it is concerned not only with the development of the spindle but also with the formation of plasmatic membranes. The predominance of kinoplasm over trophoplasm is one of the most conspicuous features of the spore-mother-cells of plants, and this may appear in a variety of ways. In certain cases a distinct perinuclear zone appears just before the first division and stains very darkly, and from the fibers of this zone and the linin of the nucleus the spindle is formed, as in *Cobaea*[13], *Lavatera*[2], *Cassia*[12], *Gossypium*[3], and other plants. On the other hand, the pollen-mother-cells of *Equisetum*[20], *Larix*[1], *Lilium*[17], and *Pisum*[4] show an entirely different condition of the cytoplasm in this respect. In these types the perinuclear zone does not exist and the origin of the achromatic figure is not so restricted. In several forms distinct fibers could be seen radiating from the nucleus toward the periphery of the cell and these fibers by folding over form a felted layer from which a multipolar spindle is later differentiated, and finally the multipolar spindle becomes bipolar by the fusion of the several poles into two. Allen[1] even suggests that this is a general process occurring in all spermatophytes. While this generalization seems not to be well founded, it does appear that we may generally refer the divisions of the spore-mother-cells in the higher plants to one type or the other. It has already been suggested (Cannon[4]) that the perinuclear zone may inhibit the formation of the spindle in the peripheral cytoplasm as it occurs in *Pisum*.

The process of mitosis as it occurs in *Micrampelis* presents some features which distinguish it from most cases. It has been pointed out above that the cytoplasm assumes a radial structure as the time approaches for the first division of the spore-mother-cell. At the same time the separation of the plasmatic membrane from the cell-wall enables the cell to assume a spherical form. The contraction of the cytoplasm is accompanied by the contraction of the nucleus so that the latter becomes angular and exceedingly distorted. Up to this time no special differentiation of the cytoplasm can be detected, but soon a few fibers can be seen running more or less parallel with the nuclear membrane; here they form a very thin reticular layer. While this change is taking place in the cytoplasm the nuclear structures are also changing. The chromatin is collecting into chromosomes and the linin appears as an almost colorless network of fine fibers which run in all directions. At this time the cytoplasm adjoining the nucleus stains a little more darkly than the rest, and as the nuclear wall dissolves, pencils of fine fibers may be seen pushing out from the nucleus into the surrounding cytoplasm. These bundles of fibers originate from the reticulum adjoining the nucleus. Soon the fibers are seen traversing the nuclear vacuole, which rapidly disappears and several poles project out from the nuclear position.

The linin fibers can no longer be distinguished as such, having been merged with those from the cytoplasm. The spindle now becomes bipolar, and the interpolar fibers are brought out in sharp contrast to the others by their stronger absorption of the stain.

Three kinds of fibers were visible: the mantle-fibers which run out from the poles of the spindle into the cytoplasm toward the equatorial region; the fibers which attach to the chromosomes and appear to draw them to the poles; and the interpolar fibers which occupy the center of the spindle and are easily distinguished throughout the anaphase and telophase. All of these except the contractile fibers are of a more or less sinuous form. The interpolar fibers are considerably thicker in their middle region, and such parts are colored quite darkly by the haematoxylon stain. During the first division the poles of the spindle do not seem to reach the peripheral cytoplasmic membrane but terminate at some distance from it (FIGURE 28). Outside of the space occupied by the spindle itself the cytoplasm is of a densely granular character, but even in relatively thin sections (3 μ) no extensions of the spindle to the "*Hautschicht*" could be seen.

The conception of an anchorage for the spindle by kinoplasmic fibers extending to the outer membrane, as expressed by Strasburger,[24] seems here hardly to be justified. It seems apparent, however, that the mantle-fibers may fix the spindle in its position by a connection with the cytoplasmic reticulum. The necessity for the fixation of the poles of the spindle in the *Hautschicht*, in order to facilitate the drawing apart of the chromosomes by contraction of the fibers, is not quite apparent. It is suggested that the interpolar fibers may act as a sort of stay, which, with a certain degree of rigidity, would facilitate the separation of the chromosomes by the contraction of the overlying fibers. The evidence at hand as to the character of the interpolar fibers certainly favors such a view.

The spindle of the second division originates like that of the first and is also multipolar in its early stages. The fibers of the cytoplasmic kinoplasm traverse the nuclear vacuole and soon obliterate it. No evidence could be found at any time of a reticulum next the nuclear wall. The spindle forms from the kinoplasmic fibers which grow in from the cytoplasm. The spindle-fibers from the first division frequently persist through the second and after the grand-daughter nuclei are formed they may be seen connected by fibers of both preceding mitoses. The spindles of the second mitosis lie in parallel planes usually at right angles to each other but often parallel. Thus the tetrads usually appear in the ordinary pyramidal arrangement but sometimes they are all in one plane. The division of the cytoplasm takes place by constriction, a narrow groove marking off the line of division. This groove deepens until the spores are fully separated.

The nuclear contents of the cells under consideration have been difficult objects for study on account of the minute size of the chromosomes. The nucleoli are often large and conspicuous and at certain stages are apparently vacuolated. In the period of development just succeeding the cutting off of the tapetal cell, the primary sporogenous cell is distinguished from its neighbors by its dense and darkly-staining cytoplasm. The nuclei of the primary tapetal and primary sporogenous cells are at first to all appearance similar. In both the chromatin is arranged about the nuclear wall in irregular masses so that the nuclear vacuole at first appears to contain only a large nucleolus. In this case, in which the spore-mother-cell is derived directed from the primary sporogenous cell, the period of growth is begun at once and the nuclear changes keep pace with the development of cytoplasmic structures. The linin of the nucleus soon becomes more apparent and its fibers may be seen passing in different directions through the nuclear vacuole. The chromatin soon shows a tendency to cling less closely to the nuclear wall and it may be seen strung along the linin network in small irregular masses. A marked increase in the quantity of chromatin is characteristic of this stage and the lines of the nuclear reticulum are thickly beaded with chromatin granules.

The reticulum now resolves itself into a continuous thread which gradually becomes more uniform. While this change is taking place all the chromatin with the nucleolus becomes crowded into one side of the nuclear vacuole and synapsis results. Often the chromatin in synapsis may be seen as a much coiled thread with many sharp convolutions, sometimes extending almost across the nucleus. During this process the nucleolus remains undiminished in size and staining properties.

At the conclusion of the synapsis period the chromatin appears in thread-like form. The longitudinal cleavage of the thread is quite plain before its transverse divisions are apparent. A considerable shrinkage of the chromatin now takes place and at the time just before the differentiation of the spindle, it has contracted to sixteen irregular masses which in some instances appear quadruple; these portions representing the division of the chromatin giving rise to the chromosomes are very small and irregular in form. The chromosomes of *Micrampelis*, by numerous counts, number sixteen in the pollen-mother-cell.

In the earlier, presynaptic condition of the chromatin an interesting condition was observed. Instead of forming a single thread, a condition such as appears in FIGURE 33 is often apparent. The thread appears to be doubled and the chromatin-masses occur frequently in pairs. Overton[21] has described the same features in *Thalictrum purpurascens*, and Cardiff[5] finds it the regular condition in *Acer platanoides* and several other plants. In *Thalictrum* the number of the chromatin-masses agrees with the number of somatic chromosomes and the inference is that these presynaptic masses of chromatin which enter synapsis in pairs are the chromosomes of the original mother-cells (archesporium). In *Micrampelis* the double thread in a presynaptic condition is not always easily demonstrated, but in certain cases appears distinctly. The chromatin soon becomes massed around the nucleolus and synapsis results. In this condition the identity of the nuclear structures is entirely obscured, the chromatin forming a dense, darkly staining mass. It is difficult to believe that this appearance in preparations of beautifully fixed material is purely an artifact, and the opinion is gaining ground that the condition of synapsis represents the final fusion of hitherto only associated elements of different parentage, a view which agrees with other facts commonly observed during the division of the spore-mother-cell. Synapsis in the material here under consideration differs in no essential from the condition usually described, so far as could be determined. At the close of the period of synapsis the chromatin emerges in the form of a much coiled thread which shows a distinct longitudinal division (FIGURE 37). A transverse segmentation is soon apparent (FIGURES 39, 40, 41), and the chromosomes thus formed contract into short, thick, and irregular masses (FIGURE 42). As this change is taking place the nuclear wall is becoming irregular and the incipient stages of spindle-formation appear in the cytoplasm. The evidence of true tetrad formation is not very abundant at this point, but in certain cases a double division of the chromosomes appeared to be quite clear (FIGURES 43, 44). The chromosomes at this stage become so nearly isodiametrical that it becomes practically impossible to distinguish between a longitudinal and a transverse division of the chromosome.

The first division is clearly of heterotypic form. The chromosomes appear united in the form of rings in the metaphase of the division (FIGURES 46, 47). The spindle fibers are attached to opposite sides of the ring and the separation takes place midway between the points of attachment of the spindle-fibers. The relation of the chromosomes to the fibers appears in FIGURE 50. After the separation of the chromosomes in the early anaphase of the division they round off into almost spherical bodies to which are attached certain bundles of spindle-fibers. In a transverse section of the spindle these can readily be seen and correspond in number to the chromosomes. The chromosomes during the anaphase can be readily counted and number sixteen (FIGURES 51, 52), a number found by Strasburger[23] and Guignard[11] in certain orchids. Owing to their minute size and the compact condition of the mitotic figure in the vegetative cells the number of chromosomes could not be determined, but it appears to be easily more than sixteen.

As the chromosomes draw closer together toward the apex of the spindle they become crowded together and lose their identity, so that as the daughter-nuclei are organized in the telophase they can no longer be distinguished from one another. They present the appearance of having become fused into a spireme (FIGURES 53, 54), which finally resolves itself into a reticulum, with a tendency toward pairing of the most conspicuous chromatin masses (FIGURE 55). This appearance is only transient, however, as the nucleus passes quickly into the prophase of the second division, when the chromatin appears in dense masses, at first angular and connected with many radiating fibers (FIGURE 56), later rounded off and peripherally disposed in the nuclear vacuole (FIGURES 57, 58).

The second division shows a thick mass of chromatin on a narrow spindle. The chromosomes here diverge in the anaphase and migrate to the poles of the spindle as two compact masses in which the form and relations of the chromosomes are entirely obscured. Only in the late telophase do the chromosomes separate from one another and distribute themselves in the newly formed nuclei (FIGURE 63). The chromatin masses soon became distributed along the threads of the nuclear reticulum (FIGURE 64), which becomes much more open and enlarged with the development of the spore.

Most of the material used in this study was fixed in an acetic acid-alcohol mixture, consisting of one part glacial acetic acid and two parts 70 per cent. alcohol, which in most cases gave excellent results. The material was preserved in 85 per cent. alcohol, cleared in xylol, and embedded in paraffine. Other methods were also used, particularly in the study of *Micrampelis*, where the peculiar cytoplasmic conditions suggested the advisability of trying different reagents. In this case accordingly three methods of fixation were used viz., acetic-alcohol, sublimate acetic mixture, and the weaker Flemming solution. The stains employed were Haidenhain's iron haematoxylon, sections counterstained with Bismarck brown, Flemming's safranin-gentian-violet-orange, also cyanin-erythrosin and fuchsin-iodin-green combinations. Particular attention was given to the rod-like bodies in the cytoplasm of *Micrampelis* and the different stains were tried on the material of each fixation. In order that conditions might be otherwise the same, certain clusters of flowers of various stages of development were embedded and sectioned, forming several series of ten to fifteen slides, which were numbered in their proper sequence, and different stains applied to slides of alternate numbers in the series. In the material fixed with acetic-alcohol the rod-like bodies appeared when the preparations were stained with iron-haematoxylon or with safranin-violet-orange. When fixed with sublimate-acetic (1 per cent. acetic) mixture the same cytoplasmic structures appeared as in the preceding case, if stained with iron-haematoxylon, safranin-violet-orange or cyanin-erythrosin combinations. In material fixed with Flemming's solution they were found only when stained with the safranin-gentian-violet and orange G. The sections were cut from 2μ to 6μ in thickness.

When by such manipulations the pollen-mother-cells of the same anthers were treated with different stains as well as those of different anthers and different fixation, the chances that the unusual structures observed in the cytoplasm are due to the reagents is minimized.

SUMMARY AND CONCLUSIONS

1. The development of the pollen was followed in several forms. In each case the layer of cells immediately below the epidermis in each anther lobe divides by a periclinal wall to form primary tapetal and primary sporogenous cells. The former again divide to form two or three layers in the wall of the pollen-sac, the latter undergo repeated divisions in the case of *Fevillea* to form a mass of pollen-mother-cells, but in *Melothria*, *Micrampelis* and *Cyclanthera* no such divisions were observed. In these features the development of the sporogenous tissue and the pollen is essentially the same as described for other members of the *Cucurbitaceae* by Mirbel, Warming and others, and is in accord with the condition found in most of the seed plants.

2. The division of pollen-mother-cells was given special attention in *Micrampelis*. The principal feature of interest here is the presence of certain darkly-staining, rod-like bodies in the cytoplasm. These appear before the mother-cells have reached their full size and persist for some time, gradually breaking up as the cell prepares for its first mitosis and appearing to resolve themselves into many minute granules which stain darkly. These granules become more conspicuous between the first and second mitoses and gradually disappear as the spore approaches maturity. No evidence was gathered as to their function, though they seem to be of kinoplasmic origin. In this they are extranuclear in origin and are not derived from the nucleolus as has been observed in the case of *Larix* and some other forms.

3. The cytoplasm appears to be distinctly fibrillar. In certain cases the fibers of the reticulum are apparently stretched in lines tangential to the nucleus during the period of its rapid expansion. As the time of the first division approaches the cytoplasm presents the aspect of fibers radiating from the nucleus. The fibrillar aspect disappears with the formation of the spindle.

4. The spindle originates partly from the linin of the nuclear reticulum but mostly from the thin weft of fibers which appears about the nucleus as its wall is gradually dissolved. As the form of the nucleus changes from spherical to angular the spindle-fibers are collected into numerous pencils pointing different directions. These gradually collect to form a bipolar spindle which stands in the center of the large mother-cell and has no apparent connection with the plasmatic membrane. The form of the spindle is narrow and sharply pointed, some of its fibers spreading far out into the cytoplasm.

5. The chromatin in early stages in the development of the spore-mother-cell is inconspicuous, but as the time for division approaches it becomes distributed in paired masses through the nuclear reticulum. The masses are regarded as representing chromosomes. They finally become merged into a compact mass as synapsis approaches.

6. Synapsis here seems to be a normal phenomenon in the life of the cell. The chromatin becomes massed about the nucleolus at one side of the nuclear vacuole. From this condition it merges from a coiled thread split longitudinally, which soon segments into sixteen double pieces. These pieces become compactly massed at the equator of the spindle, contracting into rounded masses. The separation of the chromosomes is according to the heterotypic form of mitosis. The reduced number of the chromosomes is sixteen.

7. In the telophase of the first division the chromatin forms a thread which is gradually dissolved into small granules and masses almost as in the resting nucleus. The second mitosis is inaugurated by the reassembling of this chromatin into irregular masses, which become more evenly rounded and divided into two in the ensuing metaphase. Owing to the size and rounded form of the chromosomes, the characters of a homotypic mitosis were not discernible, but the appearance of the figure is essentially different from that of the first mitosis. In the telophase of the second division the chromatin distributes itself in the form of a beaded network which persists long into the development of the spore.

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Explanation of plates 17-21

PLATE 17. *Micrampelis*

1. Transverse section through part of young anther showing the formation of primary tapetal and primary sporogenous cells. × 720.

2. Longitudinal section through somewhat older anther. The primary sporogenous cells have become the spore-mother-cells. × 720.

3. Transverse section through anther of still later stage. Spore-mother-cells usually in a single row in each locule of the anther. × 720.

4. Second division of spore-mother-cell. Tapetum and anther-wall. × 720.

5. Microspore, tapetum, and anther-wall. × 720.

6. Later stage of same. × 720.

7. First division of spore nucleus. × 562.

8. Division of spore nucleus completed. Generative cell near the wall. × 660.

9. Mature pollen grain. Generative cell elongated. Nucleus occupies most of the cell, and differentiation obscure. Vegetative nucleus to the right. × 660.

PLATE 18

Cyclanthera

10. Vertical section through one side of the androecium, showing division of hypodermal cell in the formation of primary tapetal and primary sporogenous cells. × 480.

11. Slightly later stage of the same. To the right are shown initial sporogenous and tapetal cells, *s* and *t* respectively. × 480.

12. Spore-mother-cells in synapsis stage. × 420.

13. Spore-mother-cells approaching first mitosis of mother-cell. × 480.

14. Mitosis of mother-cell. × 480.

15. Microspores and anther-wall. × 480.

Fevillea

16. Vertical section through anther rudiment. × 480.

17. Transverse section through young anther. Spore-mother-cells shaded ; tapetum not yet differentiated. × 480.

18. Later stage of same. Tapetum, T. × 480.

19. Section through part of anther showing spore-mother-cells in synapsis. Tapetal cells strongly developed. × 480.

20 and 21. Successive stages in microspore formation. Multinucleate tapetal cells. × 480.

PLATE 19. *Micrampelis*

Mitosis of pollen-mother-cells. Figures are drawn with an Abbé camera lucida. Lens combination Zeiss 2 mm. immersion objective and compensating ocular 18. Magnification of figures 1,172 diameters.

22. Normal resting cell.

23. Appearance of rod-like cytoplasmic bodies.

24. Synapsis. Cytoplasmic fibers tangent to nucleus. Disappearance of rods and appearance of darkly staining granules.

25, 26. Contraction of nucleus and preparation for division.

27. Showing origin of spindle.

28–30. Metaphase, anaphase, and telophase of first division.

31. Mother-cell showing nuclei resulting from the first and second divisions. The section includes but three of the four nuclei.

32. Young microspore.

PLATE 20. *Micrampelis*

Plates 20 and 21 deal chiefly with the changes taking place in the nuclear structures during the first and second divisions of the pollen-mother-cells of *Micrampelis*. Magnification 2,200 diameters.

33. Relatively early condition of the nucleus in mother-cell.

34. A condition somewhat later than 33. Before synapsis.

35. Synapsis.

36. Conclusion of synapsis.

37. Slightly later than 36. Spireme emerging from synapsis and showing longitudinal cleavage.

38. Double chromosomes as seen after transverse divisions of the spireme.

39–41. Appearance of the nuclei at the stage showing transverse divisions of the spireme.

42, 43. Showing condensation of the chromosomes. It is at about this time that spindle formation begins to be apparent in the cytoplasm.

44. Appearance of some of the chromosomes taken from various nuclei at about the same stage as shown in figure 43.

45. Metaphase of first division.

48. Anaphase of first division showing form of chromosomes and relation of mantle-fibers to the same. Interpolar fibers stain darkly.

49. Section through mantle-fibers attached to chromosomes; sixteen in number.

PLATE 21. *Micrampelis*

46, 47. Metaphase of first divisions; heterotypic form.

50. Chromosomes as they appear in the metaphase of the first division attached to the fibers.

51, 52. Chromosomes as they appear in the anaphase, numbering sixteen.

53, 54. Formation of spireme in telophase of first division.

55. A condition of the nucleus reached at the conclusion of the first division.

56, 57. Prophase of second division.

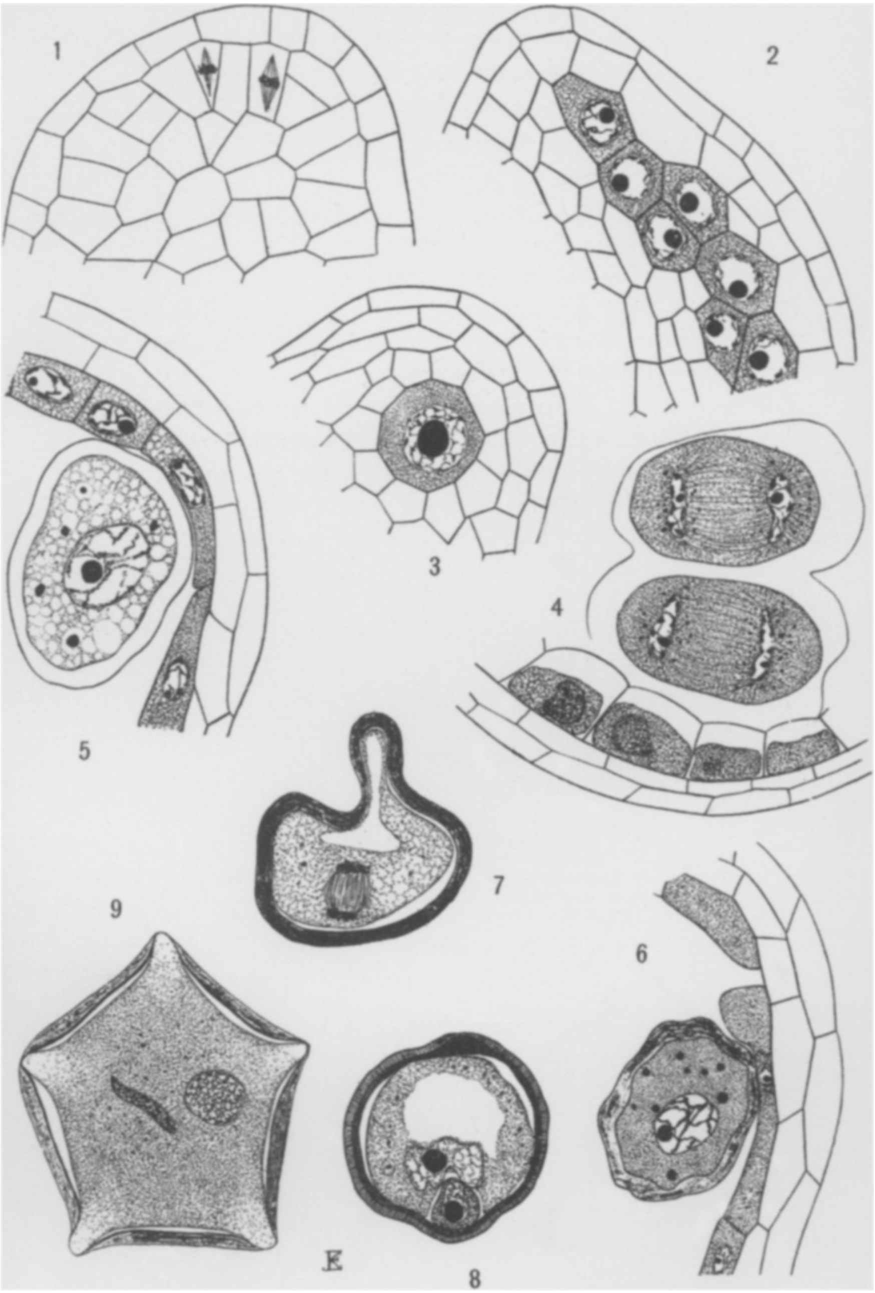
58, 59. Showing persistent spindle between nuclei in prophase of second division.

60–62. Anaphases of the second division.

63. Daughter-nucleus in telophase of the second division.

64, 65. Transition from condition shown in 63 to the reticulate resting nucleus.

66–68. Showing transformation from dark cytoplasmic fibers to the extranuclear nuclei.



1-9. MICRAMPELIS

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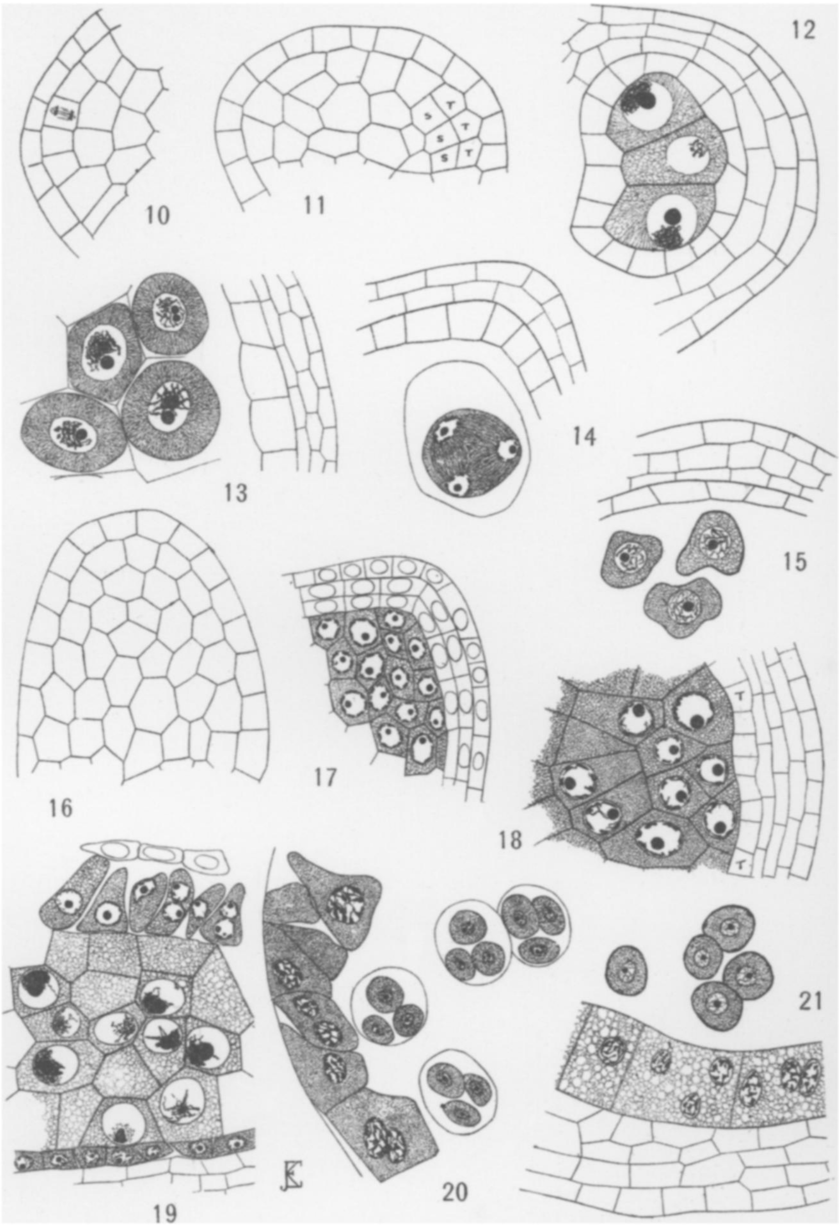
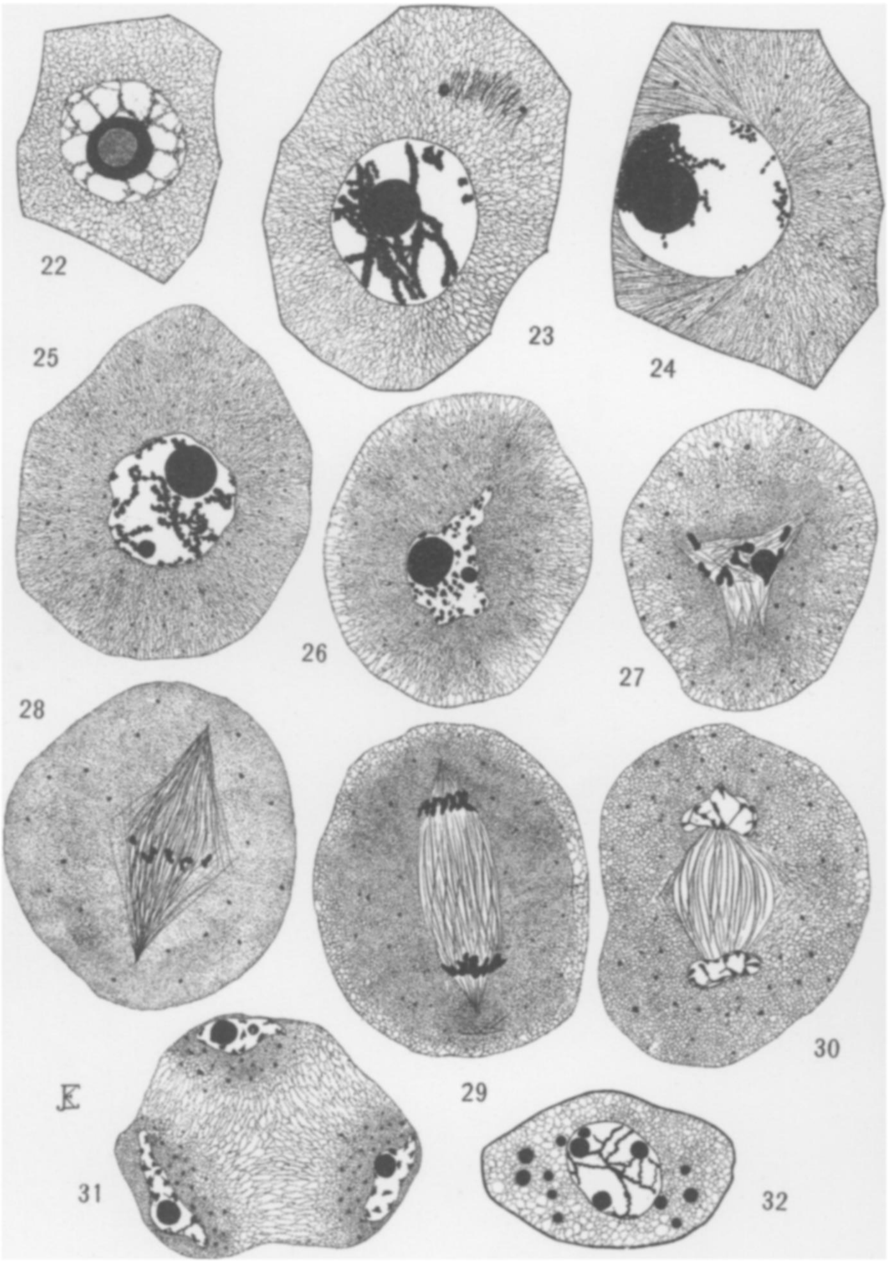


10-15. CYCLANTHERA 16-21. FEVILLEA

HELIOTYPE CO., BOSTON.



22-32. MICRAMPELIS

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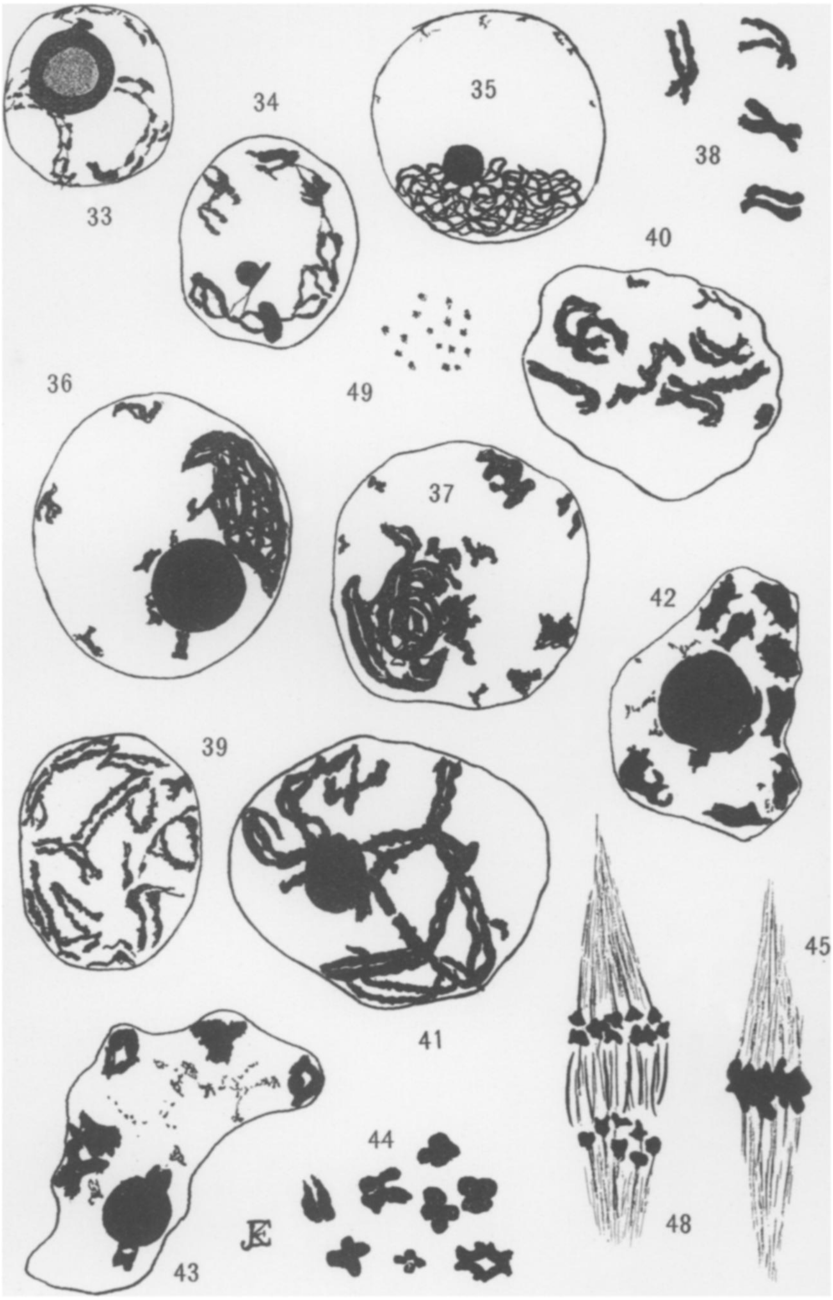

33-49. MICRAMPELIS

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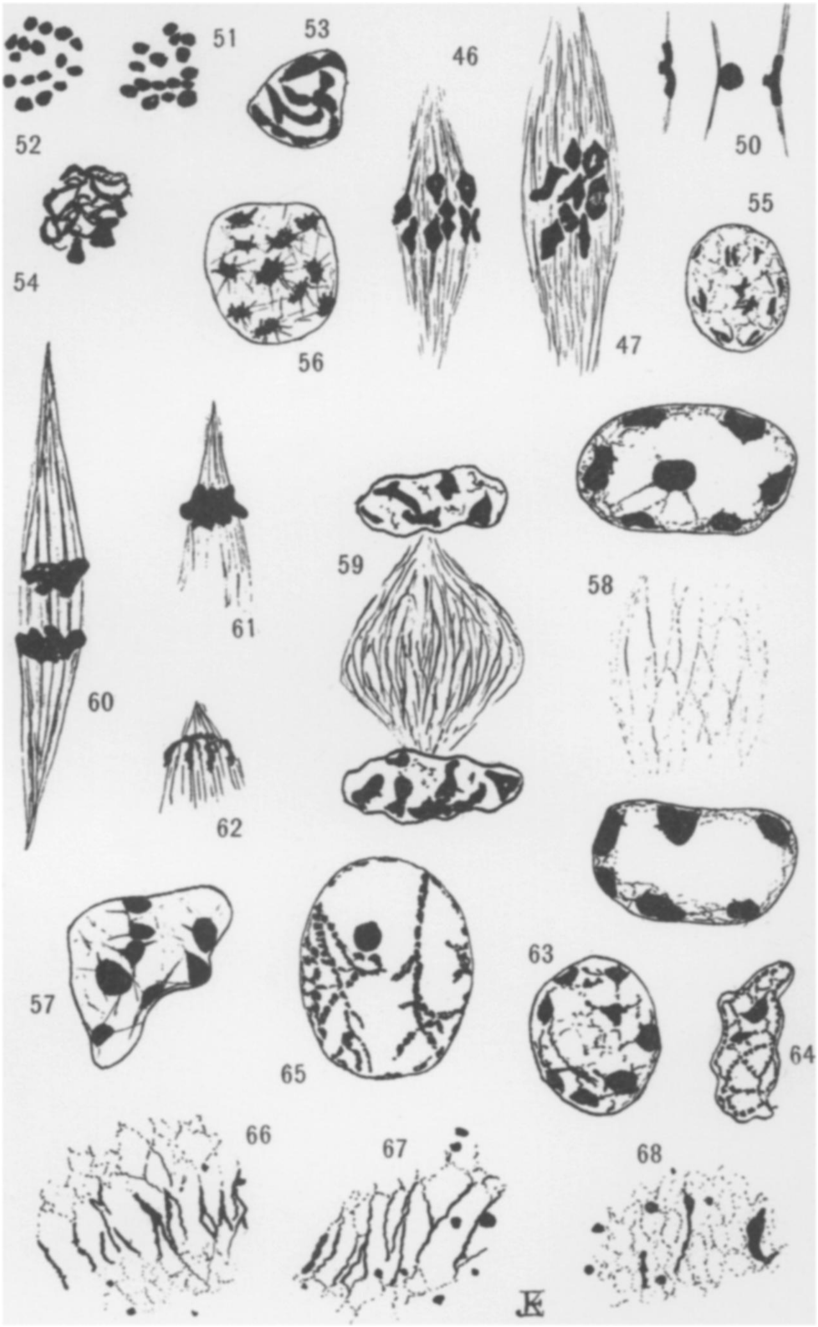

50-68. MICRAMPELIS

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